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(54) A METHOD FOR PREPARING HELIOMYCIN

(71) We, VSESOJUZNY NAUCHNO-ISSLEDOVATELSKY INSTITUT PO IZYSKANIJU NOVYKH ANTIBIOTIKOV AKADEMII MEDITSINSKIKH NAUK SSSR, 11, Bolshaya Pirogovskaya ulitsa, Moscow, Union of Soviet Socialist Republics (U.S.S.R.), A State Enterprise organised and existing under the laws of U.S.S.R., do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement: —

This invention relates to a method of preparing the antibiotic heliomycin which may be used as a 4 per cent ointment for treating burns, various forms of pyodermitis and in cosmetic medicine owing to its antibacterial and vasoconstrictive action.

A method is known by which heliomycin is produced by the culturing of *Actinomyces flavochromogenes* var. *heliomycini*. The starting culture is grown by the submerged technique in a medium containing (in per cent by weight) soya bean flour, 1.0; starch, 5—2.0; primary potassium phosphate, 0.1; sodium chloride, 0.3; calcium carbonate, 0.3; sperm oil, 0.1; water to make 100 per cent.

The same culture medium can be used for growing the seeding mycelium.

The mycelium is separated from the culture fluid by filtering and the antibiotic is then extracted from the mycelium with acetone. The moisture content of the mycelium is about 60 per cent by weight. The extraction is repeated four times, taking three litres of acetone per kilogram of damp mycelium. The first extract is discarded as having low potency, while the second and the third extracts are mixed with a double volume of distilled water. The fourth extract is used for the second extraction of mycelium obtained in the next fermentation.

The resulting crystalline precipitate of heliomycin is separated on a filter and washed with distilled water. If the crude product contains oil from the medium, it will dry with difficulty. Hence another wash with petroleum ether is required.

The yield of crude heliomycin is three per cent by weight calculated with reference to moist mycelium. The crude preparation contains from 80 to 85 per cent by weight of heliomycin.

The disadvantage of the known method for preparing heliomycin by the culture of *Actinomyces flavochromogenes* var. *heliomycini* is the great variability of the strain. During the frequent re-seeding and lengthy storage, the culture develops into more productive and less productive variants. The greatest activity with respect to accumulating the antibiotic in the mycelium is inherent in the variants whose colonies, when grown on a solid organic medium, have no aerial mycelium, and when grown on a mineral medium. have but meagre aerial mycelium and liberate reddish brown pigment. For these reasons the yield of the antibiotic is low (3 per cent by weight with reference to the moist mycelium, even with the utilization of active variants of the culture *Actinomyces flavochromogenes* var. *heliomycini*).

An object of the present invention is to obviate or mitigate the disadvantages of the aforesaid method.

According to the present invention there is provided a method of preparing the antibiotic heliomycin, comprising aerobically cultivating a heliomycin-producing strain of *Actinomyces variabilis* var. *roseolus* in an aqueous, nutrient medium containing assimilable sources of nitrogen, carbon and mineral salts nutrient medium separating the thus formed mycelium and extracting the heliomycin therefrom.

[*Price 25p*]

The new strain of *Actinomyces variabilis* var. *roseolus*, is characterized by the following physiological and morphological properties: when grown on Hause's mineral medium No. 1 it produces ample aerial mycelium of grey colour; the substrate mycelium is colourless; the medium is coloured faint pinkish brown or violet-brown; the sporangiophores are spiral, the spirals having 2—4 coils and the spores are covered with long pili; when grown in Hause's organic medium No. 2, the aerial mycelium is first creamy in colour, then it turns grey, while the substrate mycelium is from yellowish brown to dark brown in colour; when grown in Czapek and Lindenbein's medium, the aerial mycelium is ample and grey in colour, the substrate mycelium is brown; on glucose-asparagine medium the aerial mycelium is scarce, first creamy in colour, then grey; the substrate mycelium is yellowish brown, the medium is colourless; on liquid nutrient media at temperatures of 28°C it readily assimilates lactose, galactose, moderately assimilates glucose and poorly assimilates dulcitol. The strain is isolated from soil.

Any embodiment of the present invention will now be described by way of illustration.

Strain No. 6383 of *Actinomyces variabilis* var. *roseolus* which is used as the starting culture, differs from strain No. 2915 of *Actinomyces flavochromogenes* var. *heliomycini* in that it forms brown substrate mycelium and soluble pigments (pinkish brown or violet brown) when grown on synthetic media, and also in that ample quantities of spores are borne by the aerial mycelium grown on these media.

The starting culture is grown submerged in a soya-starch medium. The same medium can also be used for the productive fermentation.

The antibiotic is contained in the mycelium, which is separated from the culture fluid by filtration. The antibiotic is extracted with acetone from the mycelium which contains about 60 per cent by weight of moisture. The mycelium is extracted four times each with three litres of acetone per kilogram of damp mycelium. The potency of the first extract is low and it is therefore discarded. The second and the third extracts are combined and then mixed with twice their volume of distilled water to precipitate the heliomyces.

The fourth extract is used for the second extraction of mycelium from the next fermentation. The precipitated crystals of heliomycin are separated on a filter and washed with distilled water. If the crude antibiotic contains oily compounds it dries with difficulty and should therefore be given an additional wash with petroleum ether. The yield of crude antibiotic is 4 per cent by weight calculated with reference to the damp mycelium.

The crude preparation contains 80 to 85 per cent by weight of heliomycin calculated with reference to a chemically pure crystalline preparation.

The advantage of the proposed method lies in the utilization of a new and more productive micro-organism, viz., the strain No. 6383 of *Actinomyces variabilis* var. *roseolus*, which provides a yield of crystalline heliomycin of up to 4 per cent by weight with respect to moist mycelium.

Example

The spores are prepared by growing strain No. 6383 of *Actinomyces variabilis* var. *roseolus* in test tubes on a synthethic Hause's medium No. 1 slant for ten days at a temperature of 28°C. The inoculum is prepared by growing the spores in 500 ml Erlenmeyer flasks containing 100 ml of culture medium of the following composition, in per cent by weight:

soya bean flour		1.0
starch		1.0
sodium chloride		0.3
calcium carbonate		0.03
primary potassium phosphate		0.1
water	to make	100 ml

The pH of the medium after sterilization is 7.0.

The seeding mycelium is grown in the flasks on reciprocating shakers (200 rpm) for two days at a temperature of 28°C.

Then the inoculum (5 per cent by weight, or 400 ml per tank) is introduced into seeding tanks of 45 litre capacity containing 20 litres of nutrient medium of the following composition, in per cent by weight:

soya bean flour		1.0
starch		1.0
sodium chloride		0.3
calcium carbonate		0.3
primary potassium phosphate		0.1
sperm oil		0.1
water	to make	100 ml

The pH of the medium after sterilisation is 7.0.

The medium before inoculation has been sterilised for 45 minutes at a temperature of 120°C. The culture is fermented at a temperature of 28°C, a pressure of 0.3 to 0.5 atm, and an aeration rate of one litre of air per litre of the medium per minute. The contents are stirred continually at 300 rpm.

The fermentation is continued for 48 hours and the grown culture is discharged into 500-lit fermentation tanks to inoculate the nutrient

medium of the following composition, in per cent by weight:

soya bean flour	1.0
starch	2.0
sodium chloride	0.3
calcium carbonate	0.3
primary potassium phosphate	0.1
sperm oil	0.1
water to make	100

The pH of the medium after sterilization is 7.0.

The medium is sterilized for 43 minutes at a temperature of 120°C. The fermentation tanks are inoculated with 20 litres of the seeding material per 300 litres of the culture medium, which is about 5 to 7 per cent by weight of the seeding material.

The fermentation is carried out at a temperature of 28°C and a pressure of 0.3 to 0.5 atm. The air is sparged through the liquid at a rate of one litre per litre of medium per minute. The contents are continually stirred at 300 rpm.

The fermentation is continued for 96 to 120 hours.

The 300 litres of the fermented broth are passed through a filter to separate about 30 kg of moist mycelium containing about 60 per cent by weight of moisture.

The mycelium is extracted with acetone four times. Each time the volume of the acetone is three times (90 litres) the weight (30 kg) of the moist mycelium. The first extract having a low potency is discarded, and the second and the third extracts are combined and mixed with twice their volume of distilled water (360 litres). The resulting precipitated crystalline heliomycin is separated on a filter, and washed with distilled water. If the crude antibiotic contains oily impurities, it dries with difficulty and therefore it is given an additional wash with petroleum ether, in an amount of 5 to 10 volumes with respect to the crystalline crude antibiotic.

The 30 kg of moist mycelium (60 per cent by weight of moisture) yield 1,200 g of crude antibiotic containing from 80 to 85 per cent by weight of heliomycin, calculated with respect to chemically pure crystalline heliomycin.

WHAT WE CLAIM IS:—

1. A method of preparing the antibiotic heliomycin, comprising aerobically cultivating a heliomycin-producing strain of *Actinomyces variabilis* var. *roseolus* in an aqueous nutrient medium containing assimilable sources of nitrogen, carbon and mineral salts, separating the thus formed mycelium and extracting the heliomycin therefrom.

2. A method as claimed in claim 1, wherein the strain of *Actinomyces variabilis* var. *roseolus* is characterised by the following physiological and morphological properties: when grown on Hause's mineral medium No. 1 it produces ample aerial mycelium of grey colour; the substrate mycelium is colourless; the medium is coloured faint pinkish brown or violet-brown; the sporangiophores are spiral, the spirals having 2—4 coils and the spores are covered with long pili; when grown in Hause's organic medium No. 2, the aerial mycelium is first creamy in colour, then it turns grey, while the substrate mycelium is from yellowish brown to dark brown in colour; when grown in Czapek and Lindenbein's medium, the aerial mycelium is ample and grey in colour, the substrate mycelium is brown; on glucose-asparagine medium the aerial mycelium is scarce, first creamy in colour, then grey, the substrate mycelium is yellowish brown, the medium is colourless; on liquid nutrient media at temperatures of 28°C it readily assimiliates lactose and galactose, moderately assimilates glucose and poorly assimilates dulcitol.

3. A method for preparing heliomycin according to claim 1, substantially as hereinbefore described and with reference to the Example.

4. The antibiotic heliomycin whenever prepared by the method as claimed in any one of the preceding claims.

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